A POSSIBLE ROLE OF ASCORBATE IN BORON DEFICIENT RADISH

(*RAPHANUS SATIVA* L. CV. CHERRY BELLE)

Theresa D. Sedlacek

Thesis Prepared for the Degree of

MASTER OF SCIENCE

UNIVERSITY OF NORTH TEXAS

August 2001

APPROVED:

Don W. Smith, Major Professor
Gerard A. O'Donovan, Committee Member
Mark S. Shanley, Committee Member
Earl G. Zimmerman, Chair of the Department of Biology
C. Neal Tate, Dean of the Robert B. Toulouse School of Graduate Studies

Sedlacek, Theresa D., A Possible Role of Ascorbate in Boron Deficient Radish (*Raphanus sativa* L. cv. Cherry Belle). Master of Science (Molecular Biology), August 2001, 53 pages, 2 tables, 8 illustrations, references, 127 titles.

The most apparent symptom of boron deficiency in higher plants is a cessation of growth. Deficiency causes a reduction in ascorbate concentration and the absorption of nutrient ions. Addition of ascorbate temporarily relieves deficiency symptoms. In boron sufficient plants the addition of ascorbate to media causes an increased uptake of nutrients.

In an attempt to discover if ascorbate addition to deficient plants causes increased ion uptake, radish plants were grown hydroponically in four different strengths of boron solution. A colorimetric assay for phosphorus was performed both before and after supplementation. Results, however, were inconclusive.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

INTRODUCTION

Boron is an essential micronutrient for vascular plants (Warington, 1923), some marine algal flagellates and diatoms (Lewin, 1965, 1966), and heterocystous cyanobacteria. The latter does not require boron (B) if grown on fixed nitrogen. In the absence of this nutrient cyanobacteria need boron for the production of heterocysts which are capable of nitrogen fixation (Mateo et al., 1986). Furthermore, boron is required for the legume-*Rhizobium*-symbiotic process (Bolaños et al., 1994). To date , no nutritional requirement in animals has been proven, although it may have beneficial effects in calcium metabolism and bone development in postmenopausal women (Nielson et al., 1987). The proof that boron is an essential nutrient for higher plants is often incorrectly credited to Agulhon (1910, as cited in Loomis and Durst, 1992). He performed quantitative analyses for boron content on some 30 plant tissues from different species. He also tested the effect of borate fertilizer in relation to plant growth. His conclusions were: 1) boron was ubiquitous, 2) boron content varied between tissues and species, 3) borate stimulated the growth of some plants and was therefore beneficial, 4) since boric acid was beneficial and cheap, it could be utilized as an agricultural fertilizer, and 5) boron was not essential for plants (Loomis and Durst, 1992).

Mazé (1915,1919, as cited in Loomis and Durst, 1992) claimed that boron was an essential plant requirement. However, he also claimed that fluorine, iodine, and aluminum were also essential. According to Loomis and Durst (1992) , Mazé's experiments were not well controlled or extensive. Furthermore, his nutrient solution did

not contain molybdenum, copper, or nickel salts—all essential plant micronutrients. They concluded that the growth he noted on the addition of borate, fluorine, iodine or aluminum salts was probably due to copper contamination.

Finally, in 1923, Warington demonstrated that boron was an essential nutrient in broad bean. She also showed that boron was needed throughout the life cycle of the plant. However, she was unable to prove it necessary for rye or barley nutrition. That distinction fell to Sommer and Lipman (1926), who developed better techniques for producing distilled water, filtering air, and purifying nutrient salts. They demonstrated a boron requirement for barley and six non-leguminous dicots: sunflower, cotton, buckwheat, castor bean, flax, and mustard. Other researchers extended these studies to other plant species resulting in the recognition today that boron is an essential nutrient for vascular plants (Loomis and Durst, 1992).

Boron Chemistry

Boron is a very hard nonmetal, which is not very reactive. It is a solid at room temperature (Kask and Rawn, 1993). As a metalloid, it possesses properties intermediate between those of metals and nonmetals. The boron atom has three valence electrons (Loomis and Durst, 1992).

In nature, boron occurs as a water-soluble compound, sodium tetraborate, $Na_2B_4O_7(H_2O)_{10}$. This hydrated salt of tetraboric acid, $H_2B_4O_7$, is commonly known as borax. When borax is exposed to aqueous sulfuric acid, boric acid, H_3BO_3, is produced. Boric acid is slightly soluble and is a weak acid. The compound is planar with trigonal units that polymerize into sheets (Kask and Rawn, 1993). At high pH the boric acid

molecule can accept a hydroxyl ion from water to form a tetrahedral borate anion (Loomis and Durst, 1992).

Boric oxide, B_2O_3, is the anhydride of boric acid. It is made by strongly heating boric acid. When boric oxide is mixed and fused with silicon, calcium, and sodium oxides, a superior glass is produced which resists breakage by both mechanical and thermal shock.. Ovenware and laboratory glassware such as Pyrex and Kimax are made of borosilicate glass (Kask and Rawn, 1993). Because of boron leaching, such glassware cannot be used for boron nutrition experiments. "Soft" or "soda-lime" glass must be used, or, alternatively, plastic (Bohnsack, 1991).

The most common B-containing mineral is tourmaline, which contains approximately 3% boron. Tourmalines are borosilicates which are practically insoluble and resist weathering (Goldberg, 1993). For this reason, the boron content of soils is generally greater than that of rocks. Calcium and sodium borates occur naturally in most soils. In some minerals, boron can substitute for tetrahedrally coordinated silicon (Si). Much of the boron in soils is probably dispersed in silicate minerals. In general, soils formed from igneous rocks are less likely to contain boron than those formed from marine sediments (Norrish, 1975, as cited in Gupta, 1993). Plant material or sediments provide most of the boron in soils.

Factors Affecting Boron Availability

Plants utilize only the water-soluble boron (usually boric acid) found in soil solution (Ryan et al., 1977). Factors which affect the availability of boron in soils are: pH, soil texture, soil moisture, and temperature (Goldberg, 1993). Increasing soil

solution pH makes boron less available to plants. It is for this reason that liming of acid soils to adjust pH can produce B deficiency in plants. Historically, this result of liming has been called "B fixation" (Fleming, 1980, as cited in Goldberg, 1993). Furthermore, plants take up boron more readily at lower soil solution pH than at higher pH levels (Wear and Patterson, 1962).

Soil texture also affects boron availability. Wear and Patterson (1962) found that for identical water soluble boron content, boron uptake by plants was greatest for a coarse-textured soil (Norfolk sandy loam) and least for a fine-textured soil (Decatur silty clay). Intermediate uptake was observed for medium-textured soil (Hartsells fine sandy loam).

Boron availability decreases as soils dry. There are probably two reasons for this. The first is that under drier conditions the soil solution fraction of the soil diminishes with decreasing soil moisture. The other is that during dry conditions plants extract moisture from lower depths and the available boron content of the subsoil is less than that of the topsoil (Fleming, 1980, as cited in Goldberg, 1993).

The effect of temperature on boron availability in soils is uncertain. Research on this question has produced contradictory results. This may have been due to the fact that soils of different mineral composition were used (Goldberg, 1993).

Certain minerals in soils may adsorb boron, thus making it unavailable to plants. Among these are calcium carbonate, magnesium hydroxide, aluminum and iron oxides, clay minerals, and organic matter. Boron may also be leached from soil by flooding or in areas with high annual rainfall (Goldberg, 1993).

Boron Requirement in Plants

For the above reasons, boron is the most widespread micronutrient deficiency in plants. Goldbach (1997) has divided plants into three groups based on their boron requirements determined from dry mass (DM) leaf boron concentrations. The first group is comprised of the Graminaceous species, in particular *Poales*. These plants have very low boron requirements and hardly show deficiency symptoms during growth. Their optimum leaf concentrations are between 2 and 5 $\mu g\ g^{-1}$ DM. The second group contains the dicots and non-grass monocotyledons. Their optimum leaf concentrations range from 20 to 80 $\mu g\ g^{-1}$ DM. The third and last group comprises several latex containing plants with optimum leaf concentrations usually above 80 $\mu g\ g^{-1}$ DM. These plants have very high boron demands.

The ratio of toxic to sufficient levels of boron required for normal plant growth is smaller than for any other necessary micronutrient (Reisenauer et al., 1973). Plant boron requirements not only differ between species, but also between cultivars (Goldbach, 1997), and different parts of the plant (Lockman, 1972, as cited in Gupta, 1993). Furthermore, there are no standards for assessing boron requirements. Culture conditions vary between researches as to nutrient media (Hoagland, Arnon, Shive, etc.), potting media (hydroponics, sand, vermiculite), day length and light intensity, the part of the plant assayed, age when assayed, and method of assay (Gupta, 1993). For some deficient, sufficient, and toxic levels of boron in certain crops see Table 1.

Table 1

Deficient, Sufficient and Toxic Boron Levels for Selected Crops

mg B kg^{-1} in dry matter

Plant	Part sampled	Def.[1]	Suf.[2]	Toxic	References
Barley	Boot stage tissue	1.9-3.5	1	20	Gupta (1971a)
	Straw	7.1-8.6	21	>46	Ibid.
Beans	43-d-old plants	-----	12	>160	McKay et al. (1962)
Celery	Leaflets	20	68-432	720	Eaton (1944)
Potato	32-d-old plants	-----	12	>180	McKay et al. (1962)
Soybean	Mature trifoliate leaves at early bloom	9-10	-----	63	Woodruff (1979)
Tomato	Whole plants when 15cm tall	<12	51-88	>172	Gupta (1983)
Wheat	Boot stage tissue	2.1-5.0	8	>16	Gupta (1971a)

Def.[1]: Deficient Suf.[2]: Sufficient

Boron Deficiency: Macroscopic to Microscopic

Boron deficient plants express a variety of morphological, physiological, and biochemical effects. These effects show uniformity among species in that they occur first in growing tissues (Hu and Brown, 1994). Symptoms appear during early stages of cell development. In species whose cells produce lignin the effects appear before lignification (Loomis and Durst, 1992).

Gross morphological changes due to boron deficiency can be divided into three categories: 1) roots, 2) shoots, and 3) reproductive organs. The roots of boron deficient plants exhibit several symptoms, including inhibited extension and apical growth. Inhibited root growth can occur within 3 to 6 hours (Kouchi and Kumazawa, 1975). Thickened roots appear stubby or coral-like (Bussler, 1964). In boron deficient pea roots secondary root primordia form abnormally close to the root tip. However, these primordia seldom develop further (Sommer and Sorokin, 1928). Necrosis of both the root tip and lateral meristems effectively halts root growth (Odhnoff, 1957). Root tissues brown, especially at the root apex. Cracking of roots and brown or black heart occurs. The last is especially important in root crops such as carrot (Smilde and van Luit, 1970, as cited in Gupta, 1993), radish (Skok, 1941), and rutabaga (Shelp and Shattuck, 1987a). Cuttings taken from boron deficient plants are difficult to root (Jarvis and Booth, 1981). Furthermore, the growth of root tissue is retarded more than that of shoot tissue (Dutta and McIlrath, 1964).

Boron deficient shoots also have inhibited apical and extension growth. Apical tissues exhibit browning and necrosis (Kouchi and Kumazawa, 1975). Tissues are brittle. Stems and petioles crack and break (Yamaguchi et al., 1958 as cited in Gupta, 1993).

Stem corkiness and hollow stem disorder occur in broccoli (Shelp, 1988). Due to death of the meristem, apical dominance is lost. Plants are stunted and bushy because of new leaf growth below the necrosed stem apex (Purvis and Carolus, 1964). Die back occurs. Boron deficient leaves may have burned leaf tips (McHargue and Calfee, 1933), or exhibit chlorosis (Gupta, 1983). Leaves may also curve forward (Shelp, et al., 1987b).

The boron requirement for flower and seed production is much greater than that for vegetative growth. Mild boron deficiency may support foliage, but fail to produce flowers or seeds. Flower sterility is common in all plants (Shkolnik, 1984).

Boron deficiency affects both the male and female organs of flowers. In boron deficient maize the stamens lack sporogenous tissue and exhibit decreased pollen production and size. Low activities of several enzymes are seen, including phosphatase, catalase, invertase, and starch phosphorylase. However, the activities of amylase and ribonuclease increase (Agarwala et al., 1981). Schmucker (1934) reported a striking effect of boron deficiency on water lily (*Nymphaea*) pollen tubes. After germination, pollen tubes lengthen by growth at the tip. Upon removal of *Nymphaea* pollen tubes from stigma nectar to a boron free sugar solution, the tube tips burst within 2-3 minutes. However, Dickinson (1978) using Easter lily (*Lilium longiflorum)* pollen, reported that lack of calcium causes extensive bursting of pollen tubes, while lack of boron causes little.

More recently, Huang, et al. (2000) have reported a critical period of about 7 days in which boron deficiency causes maximal damage to wheat floret fertility. This period begins with the emergence of the flag leaf to 2-3 days after its full emergence. During this period, boron deficiency retards anther development and decreases pollen viability.

It appears that the reduction in floret fertility and grain set corresponds with the number of florets reaching meiosis during this time. They also suggested that there might be two phases of pollen development, which are sensitive to boron deficiency: 1) from premeiotic interphase through meiosis to late tetrad and 2) from mitosis I to mitosis II during which starch accumulates. The first phase of development is more sensitive to boron deficiency than the second.

The female organs of flowers are also affected by boron deficiency. In grapes, which have a high boron requirement, the pollen, pistil, and stigma develop abnormally (Scott, 1944, as cited in Shkolnik, 1984). Lewis (1980) proposed that the stigma and style require high boron levels in order to inactivate callose from the pollen tube walls. This would be accomplished by the formation of a borate-callose complex. Another observation is that in *Petunia* there is a boron gradient from the stigma through the style to the ovary. *Petunia* pollen tubes grow toward higher boron concentrations. It is possible, therefore, that boron acts as a chemotactic agent to guide pollen tube growth through reproductive tissues to the ovules (Robbertse, et al., 1990).

Even if the flowers are fertile, premature dehiscence may occur. This may also occur with immature fruit. Fruit that matures may be small in size, bronzed, and /or deformed (Goldbach, 1997).

It appears that a continuous supply of boron is needed, especially during flowering, to optimally produce seeds. When boron supply was interrupted in maize, from one week prior to tasseling until plant maturity, development of ears and kernel production was severely reduced. In some maize cultivars, unhusked ears were normal in appearance. Husking, though, revealed the absence of any kernels (Mozafar, 1987).

In black gram, boron deficiency decreased the number of pods set on and thus the yield by 40-80% depending on the cultivar (Rerkasem et al., 1988). In soybean, the number of seeds in each pod declined. Boron deficiency caused " hollow heart" in peanut seeds and to lesser extent in some soybean cultivars. " Hollow heart" is a depression in one or both cotyledons of the seed. Black gram, however, was unaffected by this symptom (Rerkasem et al., 1993).

According to Dell and Huang (1997) the best hypothesis to explain these various symptoms is that the synthesis of cell wall pectins creates a sink for boron uptake into the fruit and seed. If this demand cannot be fulfilled, seeds and fruits may abscise or abort. This reduces the competition for assimilates, resulting in seeds that are larger than normal. Abnormal growth symptoms manifest when boron supplies become limited during later development. Examples are "hollow heart" in peanut and soybean (as cited above), and deformed, cracked or bleeding mango fruit (Ram et al., 1989).

In vascular plants, boron moves through the xylem with the transpiration flow from the roots to the shoots and leaves, and accumulates in growing apical tissues. Once boron reaches the leaves it becomes fixed in the apoplast. Because its retranslocation is restricted, boron is usually termed phloem immobile (Blevins and Lukaszewski, 1998).

However, studies done in some fruit trees using stable isotope ^{10}B showed that autumnal foliar application of boron temporarily increased the boron concentration of leaves. Later, during late fall and winter, boron translocated to the bark. In the spring, it moved out of the bark and into flowers. This resulted in increased fruit set (Hanson and Breen, 1985a, Hanson and Breen 1985b, Hanson, 1991, as cited in Blevins and Lukaszewski, 1998).

Boron deficient rutabaga also exhibits limited phloem transport of boron. In this case boron is redistributed from mature leaves to roots, young leaves, and flowers (Shelp and Shattuck, 1987a). The same pattern can be observed in radish (Shelp and Shattuck, 1987b).

Boron is also translocated in the phloem of broccoli. In boron sufficient broccoli, a steep decreasing gradient of boron was observed from mature to young tissues. However, in boron deprived plants, tissue boron levels were similar in all plant parts. Broccoli plants grown in sufficient boron and then switched to zero boron exhibited a decrease in tissue boron in young tissues to about 2-3 times the level of plants grown only in zero boron treatment. The mature leaves showed a slight decrease in tissue boron level (Shelp, 1988).

Hu and Brown (1996) showed that phloem mobility of boron was species dependent. Sorbitol is a major sugar produced in almond, apple, and nectarine. In these species, foliar applied ^{10}B was transported from the leaves to nearby fruit and deposited specifically in developing tissues (hull, shell or kernel). Between old and young leaves boron concentration differed only slightly. The boron concentration of fruit tissue was much greater than that of the leaves. Very little of the ^{11}B present in the leaves at the time of foliar application was retranslocated. Sorbitol-poor species tested were fig, pistachio, and walnut. These species had much higher boron concentrations in older leaves than in young. Fruit tissue had the lowest concentration. This team subsequently isolated and characterized sorbitol-boron-sorbitol complexes from peach nectar and mannitol-boron-mannitol complexes from celery sap. Thus, the phloem transport of

boron appears to depend on the polyol or sugar transport molecules utilized by the plant (Hu et al., 1997).

Boron deficiency causes a general disintegration of the phloem and cambium. Skok (1941) noted that in boron deficient radish the tissue near the axis was unaffected. He attributed this to boron contamination or to boron present in the seed. The vascular tissues between the uninjured tissue and the outer cambium layer, however, were affected. In this area there were no normal lignified vessels. The phloem cells had disintegrated. Xylem parenchyma cells were smaller that those of boron sufficient plants.

In the cambium region, there were slightly developed vascular bundles at or near a crack in the tissue. The phloem and cambium cells had mostly disintegrated. Unlignified, but well developed vascular bundles were present in the cambium in areas where the tissue was not split. These bundles were arranged in circular areas and pronounced domes. Xylem parenchyma and ray cells were highly active throughout the region (Skok, 1941).

The presence and extent of vascular damage in boron deficient plants varies among species. Palser and McIlrath (1956) reported that there is no disintegration of phloem or cambium in boron deficient cotton. When compared to Skok's observations of damage in radish (see above) it is obvious that there is a wide spectrum of physical damage in plant response to boron deficiency.

Besides disintegration of vascular tissue, callose may deposit in the phloem of boron deficient plants. In red kidney bean heavy plugs of callose formed in the sieve

plates. In this study (van de Venter and Currier, 1977), cotton exhibited some damage in the cambium and deposition of callose in the sieve plates of petioles. Since callose is formed upon mechanical tissue damage (Currier, 1957), this may be a secondary effect of boron deficiency. However, the translocation of ^{14}C was greatly reduced in both red kidney bean and cotton (van de Venter and Currier, 1977).

Spurr (1957) observed differences in cell wall thickness in boron deficient celery. In general, most collenchyma cell walls were thinner, but those of phloem and ground parenchyma became thicker. The middle lamellae in collenchyma cells were thinner and there were fewer lamellae. In some cells the normal "corner" thickenings did not develop. Instead a uniformly thick layer of cell wall material formed.

In boron deficient tomato root tips the cell walls thickened irregularly and appeared serrated. Disorganization of the middle lamella was followed by an accumulation of vesicles in the disorganized area. The Golgi apparatus developed abnormally creating an increase in the number and size of secretory vesicles (Kouchi and Kumazawa, 1976).

Hirsch and Torrey (1980) observed the above phenomena in sunflower root cells. In addition to this, the cristae of the mitochondria became swollen and later disintegrated. Vacuoles accumulated electron-dense deposits. After 20 hours of boron deprivation, the distinction between organelles and cytoplasm was lost, probably because of progressive breakdown of membranes. By 72 hours membrane integrity was gone and the cytoplasm was disintegrating.

Perhaps the most important of these symptoms are changes observed in the cell wall and middle lamella. Originally viewed as a static, rigid box, which just happened to contain a living cell, the cell wall is now considered to be a dynamic, changing structure which is an extension of the cytoplasm (Carpita and Gibeaut, 1993).

Views of the middle lamella have also changed through the years. At first, the lamella was thought to be merely a part of the cell wall. Then it was perceived as a distinct, but separate, region adjacent to the wall. Recent discoveries have changed its structural model again to one where the lamella provides a pectin matrix that holds cellulose microfibrils together, resists turgor pressure, and through interaction with the microfibril matrix regulates the rate of cell expansion (Carpita and Gibeaut, 1993, Devlin, 1975, Nobel, 1991).

The middle lamella is composed of pectic polysaccharides. Three fundamental constituents are polygalacturonic acid (PGA), rhamnogalacturonan I (RG I), and rhamnogalacturonan II (RG II). PGA is a helical homopolymer made up of (1→4) α-D-galactosyluronic acid (GalA) units. RG I is a kinked rod-like heteropolymer of repeating disaccharide units of (1→2) α-L-rhamnosyl-(1→4) α-D-GalA (Carpita and Gibeaut, 1993). RG II is a highly substituted, branched homogalacturonan. It is characterized by unusual sugars, which are found only in RG II. Two of these sugars, apiose and aceryl acid, are the attachment points for the complex side chains to the homogalacturon backbone (Zablackis et al., 1995).

PGA chains can condense to form junction zones. This occurs when calcium (Ca) ions chelate to the oxygen atoms of unesterified galacturonosyl residues on separate PGA chains (Darvill et al., 1980, Gibeaut and Carpita, 1994). It is unknown how many chains can condense or how many unesterified GalA residues are needed to form a junction zone (Carpita and Gibeaut, 1993).

Teasdale and Richards (1990) experimented with culture media for pine cells. They found that the amount of boron needed for maximum growth rate varied depending on the concentrations of Ca and magnesium (Mg) ions in the medium. They proposed a hypothetical acceptor molecule, which was activated after binding both Ca and boron (Teasdale and Richards, 1990).

Boron is localized in the cell walls of higher plants (Hu and Brown, 1994) by forming a borate diol ester bond which cross links two RG II chains (B-RG-II) between two apiosyl residues (Kobayashi et al., 1996). Immunogold labeling with an antibody toward B-RG-II pinpoints its distribution to the inner one third of the cell wall closest to the plasmalemma. Denser labeling occurs at the junctions of cell corners. However, the RG II epitope was not detected in the middle lamella between cell walls (Matoh, 1997).

Boron-pectic complexes produced *in vitro* are pH dependent. Acidification of these gels result in liquefaction (Hu and Brown, 1994). The stability of reconstituted B-RG-II complex is enhanced by the addition of Ca ion. Also, the native B-RG-II complex contains two mol of Ca (Matoh, 1997) in addition to 2.1 mol of B (Kobayashi et al., 1996). Thus, B-RG-II may be the acceptor molecule proposed by Teasdale and Richards.

The B-RG-II complex appears to be ubiquitous in higher plants. It has been found in 25 species distributed among 11 families representing both *Dicotyledoneae* and *Monocotyledoneae* (Ishii and Matsunage, 1996, Kobayashi et al., 1996, Matoh et al., 1996). Furthermore, species requirement for boron is correlated to the amount of pectin present in cell walls (Hu et al., 1996). Only small amounts of pectin are found in graminacious monocots (Darvill et al., 1980). Even though their boron concentrations are the lowest of all species, they exhibit no signs of boron deficiency when deprived of boron (Hu et al., 1996). The cell walls of dicots, however, contain a moderately high level of pectin (Darvill et al., 1980), have higher levels of boron in their leaves, and suffer from boron deficiency when deprived of the nutrient (Hu et al., 1996). Asparagus, a nongraminaceous monocot, has cell walls rich in pectin (Waldron and Selvendran, 1990), and is the most sensitive to boron deficiency (Hu et al., 1996).

Under boron deficient conditions the middle lamella becomes disorganized or disrupted (Hirsch and Torrey, 1980). However, the synthesis of cell wall polysaccharides is not altered (Goldbach and Amberger, 1986, Hu et al., 1996). Secretory vesicles accumulate at the plasmalemma side of the plasma membrane cell wall interface (Hirsch and Torrey, 1980). These vesicles presumably contain cell wall/middle lamella materials (Matoh, 1997).

A growing cell wall is composed of elastic and plastic elements. Elastic elements stretch when tension is applied and then upon removal of the stress return to their original shape. Plastic elements, however, either break or stretch irreversibly when tension is applied (Cosgrove, 1987). The B-RG-II complex is an elastic element of the cell wall

(Goldbach, 1997). A rapid reduction of the cell wall elasticity modulus as measured by a cell pressure probe was seen after rinsing squash roots with a relatively boron-free nutrient solution. After 20 minutes the elasticity modulus rose again. This indicated a secondary effect resulting in wall stiffening (Findeklee and Goldbach, 1996). However, the primary reduction of the elasticity modulus observed by Findeklee and Goldbach was not due to a loss of boron because cell wall boron remains constant or suffers little loss. Cytoplasmic boron, though, is drained (Hu and Brown, 1994).

Effect of Boron on the Plant Cell Plasmalemma

The B-RG-II complex explains some of the effects seen in boron deficient plants. However, this role of structural integrity does not explain all of the symptoms of boron deficiency. The plasma membrane is also affected with a concomitant change in the uptake of essential nutrients.

Pollard et al. (1977) were the first to localize boron to the cell membrane in maize root tips. Six years later Tanada (1983) confirmed this discovery using a spectrofluorometric analysis on mung bean hypocotyls, protoplasts, and protoplast membranes. The membranes had the highest boron content at 20.1 $\mu g\ mg^{-1}$ protein.

Previously, Robertson and Loughman (1974) had shown that phosphate absorption was reduced in root segments and whole plants of *Vicia faba*. Addition of boron to the deficient plants for a period of 1-2 hours before testing, restored both

absorption and metabolism of phosphate. However, the initial rates of esterification of the absorbed phosphate were not increased.

Reduced absorption of phosphate, chloride, and rubidium (a potassium analog) ions were also observed in roots and root tips of maize and *Vicia faba* (Pollard et al., 1977). The addition of boron caused a three-fold increase (40%) in ion absorption in maize in 20 minutes. A comparable two-fold increase was seen in *Vicia faba*. In the same study the activity of the KCl-stimulated adenosine triphosphatase (ATPase) in maize was examined. As the root tip membrane fractions became boron deficient the *in vitro* activity of the ATPase declined progressively.

Goldbach (1985) found reduced ^{32}P and ^{14}C-glucose uptake in boron deficient sunflower (*Helianthus annuus*) roots and carrot (*Daucus carota*) cell suspensions. He also observed that efflux rates were lower in boron deficient materials. This effect might have been due to reduced membrane permeability. Again, functions were restored in less than 1 hour after boron addition.

A fifty-percent reduction in the ferricyanide-induced net proton release was observed in boron deficient suspension cultured tomato and carrot cells. Addition of boron in the presence of auxin reversed the effect. Vanadate suppressed the proton release. This indicated that the plasma membrane proton pump was involved (Goldbach et al., 1990).

Subsequently, it was shown that boron deficiency inhibited the vanadate-sensitive H^+-ATPase and NADH-FeCN reductase activities in sunflower microsomes (Ferrol and

Donaire, 1992). However, the amount of antigenic ATPase protein as measured by immunoblotting of sunflower cell microsomes remained the same. Fluorescence anisotropy showed that 1-(4-trimethylammonium-phenyl)-6-phenyl-1,3,5-hexatriene and diphenyl hexatriene probes were immobilized to a greater extent in boron deficient cell microsomes than in boron sufficient ones. This was interpreted as a decrease in membrane fluidity (Ferrol et al., 1993). Because of this, external acidification of excised sunflower roots was also inhibited (Roldan et al., 1995).

Schon et al. (1990) found that exposure of sunflower root cell membranes to boron produced a hyperpolarization in the plasmalemma in 3 minutes. Membrane permeability to potassium increased immediately, resulting in a greater driving force for K^+ influx.

Barr et al. (1993) demonstrated that the auxin-sensitive plasma membrane NADH oxidase was also inhibited by boron deficiency. Ferricyanide-generated proton release was affected more severely than that associated with the plasma membrane H^+-ATPase, reflecting electron transport of the plasmalemma.

Morré et al. (1986) demonstrated that the auxin-sensitive plasmalemma NADH oxidase was, in fact, ascorbate free radical (AFR) oxidoreductase. Boron stimulation of AFR oxidoreductase might be responsible for keeping ascorbate reduced at the plasma membrane/cell wall interface. Both ascorbate and NADH oxidase have been implicated in plant growth processes (Blevins and Lukaszewski, 1998).

Lukaszewski and Blevins (1996) observed a decrease of up to 98% in the ascorbate concentration in root meristems of squash (*Cucurbita pepo* L.). Although the decline was in proportion to growth rate it was not related to ascorbate oxidation. The addition of ascorbate temporarily relieved boron deficiency symptoms. Cakmak and Römheld (1997) proposed that the cessation of growth in boron deficient plants could be partially explained by reduced biosynthesis of ascorbic acid and, at the same time, increased consumption of ascorbate.

The Roles of Ascorbate in Plant Growth

Ascorbate (vitamin C) plays several roles in plant physiology. It has been implicated in plant growth and the control of biosynthesis of hydroxyproline-rich proteins. As an antioxidant it helps protect against active oxygen species, both directly and indirectly through the regeneration of other antioxidants which have been oxidized (Cordoba and González-Reyes, 1994, Smirnoff, 1996). Ascorbate is an important dietary nutrient for humans, primates and guinea pigs because of the lack of a functional form of the enzyme L-gulono-1,4-lactone oxidase (Nishikimi et al., 1994). Despite this, its metabolism is poorly understood and its biosynthetic pathway has not been fully elucidated (Conklin et al., 1999, Smirnoff, 1996, Wheeler et al., 1998).

Ascorbate is capable of autoxidation at 25°C or in the presence of small amounts of copper (Cu^{+2}) at 15°C (Gonzalez-Reyes et al., 1994a). It is also oxidized by the enzyme ascorbate oxidase (AO). The role of AO in the balance of ascorbate metabolism

is unknown (Noctor and Foyer, 1998). Ascorbic acid is also oxidized in its antioxidant role as a scavenger of hydrogen peroxide (H_2O_2) and other active oxygen species (AOS). It also regenerates membrane-bound quenchers such as α-tocopherol (vitamin E) and zeaxanthin, a photoprotectant (Foyer et al., 1991).

The immediate product of ascorbate oxidation is monodehydroascorbate (MDHA) or ascorbate free radical (AFR). This radical is highly unstable and quickly disproportionates to ascorbate and dehydroascorbate (DHA). DHA reductase reduces DHA to ascorbate, using glutathione as the reducing substrate (Noctor and Foyer, 1998).

Several aspects of plant growth have been linked to ascorbate or its free radical. Exogenous ascorbate added to growing onion roots enhances the cell cycle by increasing the proportion of cells progressing from G1 to S phase. It has been suggested that this effect on mitotic time is due to ascorbate's role as a cosubstrate in hydroxyproline-rich protein synthesis (Arrigoni et al., 1977) as these proteins may be involved in cell division (Liso et al., 1984). However, it is actually the AFR produced by ascorbate oxidation rather than the parent molecule which accelerates the shift of quiescent cells into proliferation (De Cabo et al., 1993).

AFR also enhances vacuolization in plant cells. Vacuoles increase in volume, volume density, tonoplast surface, and tonoplast surface density. Other organelles, though, do not significantly change (Hidalgo et al., 1989). This increase can be linked to an AFR-induced stimulation of nutrient uptake (González-Reyes et al., 1994b). Since vacuoles provide the turgor pressure necessary for cell expansion (Cosgrove, 1987) this leads to cell elongation (Hidalgo et al., 1991).

The increased nutrient uptake by roots has been explained by the observation that AFR induces a stimulation of potassium ion (K^+) efflux, which in turn causes a hyperpolarization of the plasma membrane. This hyperpolarization could facilitate transport across the membrane (González-Reyes et al., 1992, González-Reyes, 1994). AFR also induces a drastic increase of net proton efflux (González-Reyes et al., 1992). Recently, it has been shown that AFR can act as an electron acceptor to a plant plasma membrane b-type cytochrome in ascorbate loaded plasmalemma vesicles. The cytochrome is reduced only in ascorbate-loaded vesicles, indicating that ascorbate acts as an electron donor. Since the membrane is not readily permeable to either ascorbate or its free radical these observations indicate the existence of a transmembrane electron transport system (Horemans et al., 1994). Electron transport could stimulate the plasmalemma proton pumping H^+-ATPase which could account for the large proton efflux (Smirnoff, 1996). The resulting acidification of the cell wall would support the acid growth theory, which states, in part, that a decrease in apoplastic pH activates enzymes that break cell wall bonds, allowing the expansion of a growing cell (Taiz and Zeiger, 1991).

As previously noted, ascorbate is involved in the biosynthesis of hydroxyproline-rich proteins. It is used as a cosubstrate by peptidyl-prolyl-4 hydroxylase in the hydroxylation of peptidyl proline residues (De Tullio et al., 1999). With the exception of the glycine-rich proteins, all cell wall proteins contain hydroxyproline residues. These proteins are comprised of four groups: hydroxyproline-rich glycoproteins (extensins), proline-rich proteins, arabinogalactan proteins, and solanaceous lectins which are chimeric proteins that contain extensin-like domains (Cassab, 1998). Thus, ascorbate is

involved in the biosynthesis of most cell wall proteins. The nodule cell walls of boron deficient *Phaseolus vulgaris* L. inoculated with *Rhizobium tropici* have been shown to contain no covalently bound hydroxyproline- or proline –rich proteins. Since ENOD2 mRNA was present it was thought that the problem was assembly into the cell wall (Bonilla et al., 1997). However, it might be a problem with post-translational processing.

Crosslinking of cell wall components stiffens the cell wall, thereby reducing the rate of elongation. For example, isodityrosine bonds and polysaccharide polymers link extensin molecules by diferulate bridges. Both of these linkages are formed by cell wall and /or apoplastic peroxidases (Fry, 1986). Ascorbate is found in both the symplast and apoplast and is a strong inhibitor of peroxidases (Takahama and Oniki, 1992). However, there appears to be a differential effect depending on the location of the peroxidase. Ascorbate only partially inhibits cell wall ferulic-acid-dependent peroxidase. However, ferulic acid peroxidase from apoplastic fluid is totally inhibited as long as ascorbate is present in the assay medium (Cordoba-Pedregosa et al., 1996).

Finally, ascorbate is able to perform non-enzymatic scission of xyloglucan, a cell wall polysaccharide. Xyloglucan hydrogen bonds to cellulose and may tether adjacent microfibrils. Added hydrogen peroxide and copper increase the scission rate, but hydroxyl radical scavengers inhibit the reaction. It has been proposed that ascorbate reduces O_2 to H_2O_2, and Cu^{2+} to Cu^{+}. Hydrogen peroxide and Cu^{+} react to form hydroxyl radical, which causes oxidative scission of xyloglucan (and possibly other cell wall polysaccharides). Since hydroxyl radicals are very short-lived they may act as site-specific oxidants (Fry, 1998).

Some Nutrient Deficiencies Linked with Boron Deficiency

One of the most important effects of ascorbate on plant roots is that it increases nutrient uptake. As mentioned previously, boron deficiency decreases the uptake of phosphorus and potassium. Leaves of boron deficient tobacco plants have decreased levels of nitrate, calcium, magnesium, and potassium (Camacho-Cristóbal and González-Fontes, 1999). In cotton, *Gossypium hirsutum* L., there is a reduced uptake of manganese and zinc (Ohki, 1975). The reduction of elements other than boron can lead to symptoms of other mineral deficiencies. For example, the buildup of starch in leaves that is seen in boron deficiency is also a symptom of nitrogen deficiency (Marschner, 1995). Thus, boron deficiency is not a single nutrient disorder as it creates multiple nutrient deficiencies (Mozafar, 1989).

MATERIALS AND METHODS

Experimental nutrient solutions modified after Arnon and Hoagland (Dutta, 1987, Jones, 1983) were prepared and stored in plastic ware which had been acid rinsed in 4N H_2SO_4. (The reagents and quantities are listed in Table 2). Distilled deionized water from a Milli Q Water System was used in all solutions. Stock solutions were made of the micronutrients. The macronutrient solution was prepared in two parts. Solution A contained calcium nitrate ($CaNO_3$) and iron sulfate ($FeSO_4 \cdot 7H_2O$). Solution B contained all of the other macronutrients and micronutrients, with the exception of boron. Solutions A and B were mixed and stored separately to avoid precipitation (Jones, 1983). Four hydroponics treatment solutions with boron concentrations of 0.25 mg B l^{-1}, 0.50 mg B l^{-1}, 0.75 mg B l^{-1}, and 1.00 mg B l^{-1} were mixed just before transplantation of seedlings. The pH of all solutions was adjusted to 5.9 using a Fisher Scientific Accumet Basic AB15 pH meter.

Radish seeds (*Raphanus sativa* L. cv Cherry Belle) were purchased from Home Depot. The seeds were rinsed in a very weak bleach solution, then rinsed in Milli Q water, and germinated in 9cm plastic Petri dishes between two disks of Whatman #1 filter paper. The filter paper was moistened with Milli Q water. The Petri dishes were then sealed with strips of Parafilm and germinated in a shadowed corner of a growth room. After 3 days the seedlings were transplanted to hydroponics containers.

The hydroponics pots were made from one-quart plastic freezer containers. Thirteen holes were drilled in each lid: 3 along each side, 1 in the center, and 1 each (4 total) between the center and corner holes. Parafilm was stretched across the lid and

Table 2

Formula for Modified Arnon and Hoagland Nutrient Solution

Common Name	Formula	g l^{-1} (mg l^{-1})
Solution A		
Calcium Nitrate	$(CaNO_3)_2$	0.49 g
Iron Sulfate	$FeSO_4 \cdot 7H_2O$	0.025 g
Solution B		
Potassium Nitrate	KNO_3	1.02 g
Magnesium Sulfate	$MgSO_4 \cdot 7H_2O$	0.49 g
Ammonium Phosphate	$NH_4H_2PO_4$	0.23 g
Manganous Chloride	$MnCl_2 \cdot 4\ H_2O$	1.81 mg
Zinc Sulfate	$ZnSO_4 \cdot 7H_2O$	0.22 mg
Sodium Molybdate	$Na_2MoO_4 \cdot 2H_2O$	0.09 mg
Cupric Sulfate	$CuSO_4 \cdot 5H_2O$	0.08 mg
Boric Acid*	H_3BO_3	1.00 mg

* Various aliquots of the boric acid stock solution were added last to the hydroponics solution to produce treatments of 0.25 mg B l^{-1}, 0.50 mg B l^{-1}, 0.75 mg B l^{-1}, and 1.00 mg B l^{-1}.

holes were punched through. The seedlings were inserted through the film so that the root was in the solution and the leaves were on top of the Parafilm.

Fritz Mighty III aquarium air pumps connected to Fritz 5-nozzle gang valves provided aeration. Five-millimeter rubber tubing was used for connections. Nozzles were made by circularly scoring and then snapping 1-ml plastic serological pipettes. The nozzles were inserted through the middle hole in the freezer container lids. All materials were acid rinsed before assembly.

After 3 days growth (photoperiod of 16 hours) the plants grown in 1 ml B solution were rinsed 3 times in Milli Q water to remove all traces of boron, and transplanted to pots containing boron deficient solution. After 3 days half of the plants in each solution were sacrificed to assay phosphorus content in the root, leaf, and stem portions. Reagents were mixed and the tests conducted on Whatman #1 filter paper disks as described by Jones (1998).

The remaining plants were supplemented with 1 μM ascorbate. Ascorbate stock solution was refrigerated and added to the media every 6 hours. After 24 hours the plants were assayed for phosphorus content again.

RESULTS

All of the plants exhibited boron deficiency symptoms before the first phosphorus test. The worst group was the 0.25 mg B l^{-1}. All of the plants had severely curled leaves. Some leaves had chlorosis or "burnt" browned tips. Root tips were browned and the hypocotyls had brown spots.

The 0.50 mg B l^{-1} group had similar symptoms except that a few had half-curled leaves. Fewer root tips were lost in this group, but browning of the tips and hypocotyls was still pervasive.

The 0.75 mg B l^{-1} group had a mixture of half-curled and straight leaves. Very few plants had lost their root tips.

The 0.00 mg B l^{-1} group, which had been switched from 1.00 mg B l^{-1} to deficient solution had moderately curled leaves. Some were beginning to sprout the second set of leaves (the first true leaves). The root tips and hypocotyls were browning.

After ascorbate supplementation, members of the 0.25 mg B l^{-1} group still exhibited severe boron deficiency symptoms. Leaves were curled and crumpled. Roots had died back. Most of the plants had died.

The 0.50 mg B l^{-1} set of plants showed a slight improvement. Leaves ranged from curled, to half-curled, to straight. Some were getting the second set of leaves. Others were forming the third set of leaves (the second set of true leaves).

The 0.75 mg B l^{-1} group showed the most improvement. Leaves were moderately curled to straight. The tertiary sets of leaves were forming. In general, the leaves of this group, both primary and secondary, were the largest and healthiest in terms of color, breadth, and straightness.

The 0.00 mg B l^{-1} group had a mixture of curled and moderately curled leaves. The first set of true leaves were still forming, or where they had unfolded were thinner (1mm width compared to 2-3 mm in the 0.75 mg B l^{-1} group). In general, the root tips had been lost and there were many short adventitious roots.

The colorimetric phosphorus tests were inconclusive since the intensity of the color appeared to be the same for both before and after ascorbate supplementation. See figures 1 and 2 for the before and after tests for the 0.25 mg B l^{-1} group. Figures 3 and 4 show results for the 0.50 mg B l^{-1} group. Figures 5 and 6 represent the before and after results for the 0.75 mg B l^{-1} group. And figures 7 and 8 show results for the plant group that was switched from 1.00 mg B l^{-1} solution to a 0.00 mg B l^{-1} solution.

Fig. 1 Phosphorus test before addition of ascorbate to 25% B group. 25% B = 0.25 mg B l^{-1}. R = root, L = leaf, S = stem.

Fig.1

Fig. 2 Phosphorus test after addition of ascorbate to 25% B group. 25% = 0.25 mg B l^{-1}.

R = root, L = leaf, S = stem.

Fig.2

Fig. 3 Phosphorus test before addition of ascorbate to 50% B group. 50% = 0.50 mg B l^{-1}. R = root, L = leaf, S = stem.

Fig. 3

Fig. 4 Phosphorus test after addition of ascorbate to 50% B group. 50% = 0.50 mg B l^{-1}.

R = root, L = leaf, S = stem.

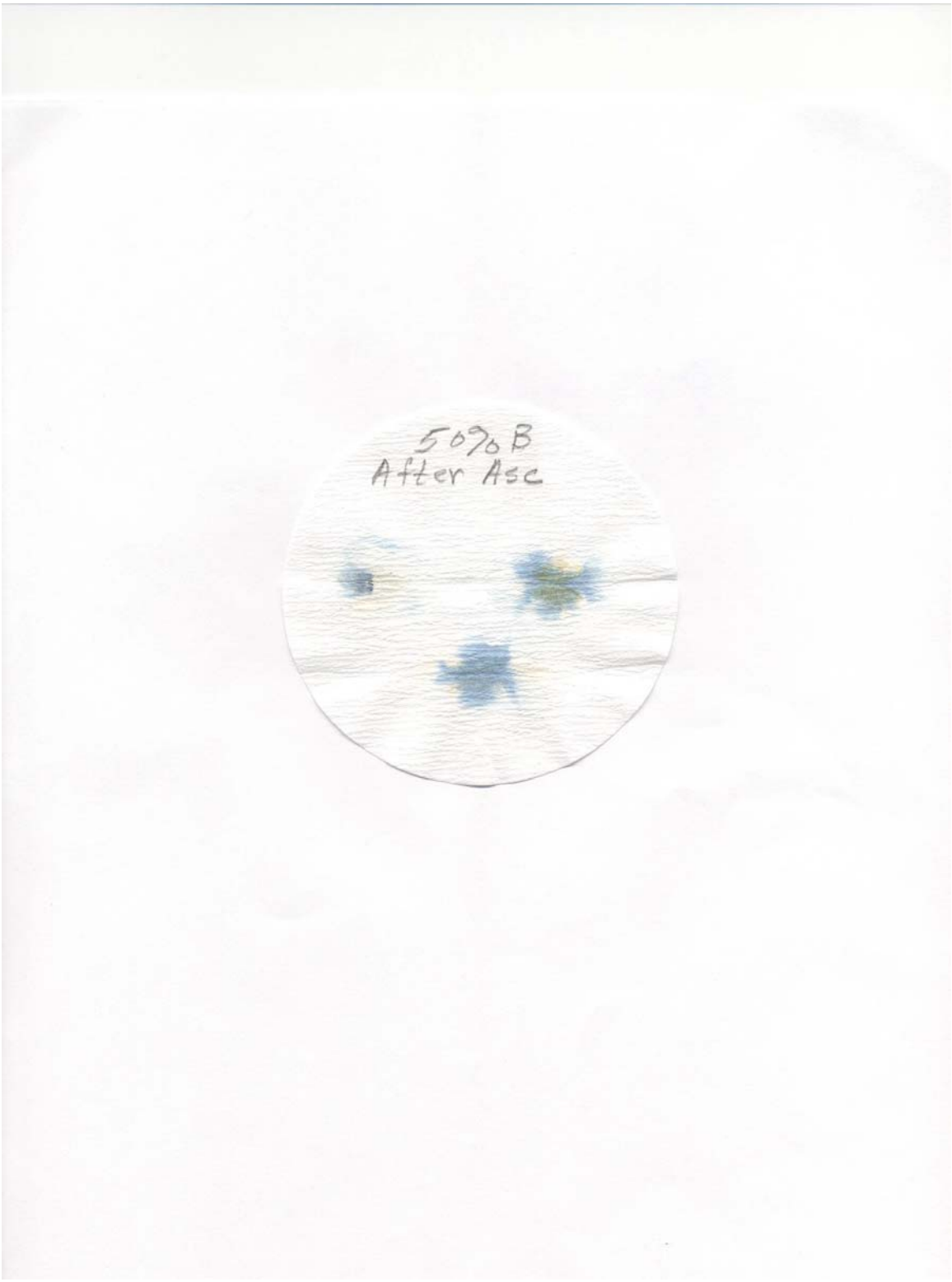

Fig. 4

Fig. 5 Phosphorus test before addition of ascorbate to 75 % B group. 75% = 0.75 mg B l^{-1}. R = root, L = leaf, S = stem.

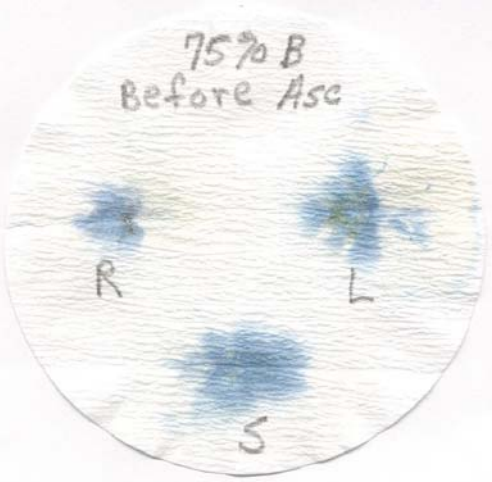

Fig. 5

Fig. 6 Phosphorus test after addition of ascorbate to 75% B group. 75% = 0.75 mg B l^{-1}. R = root, L = leaf, S = stem.

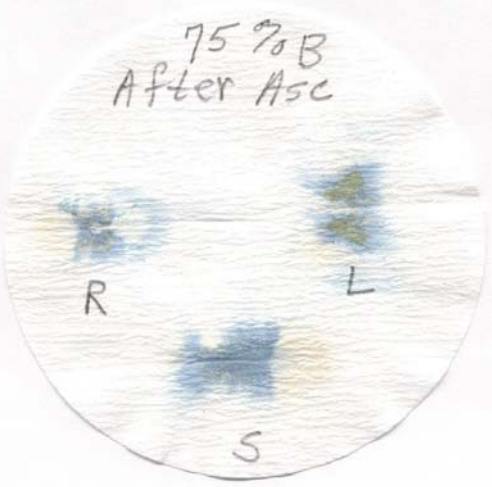

Fig. 6

Fig. 7 Phosphorus test before addition of ascorbate to 0% B group. 0% = 0.00 mg B l^{-1}.

R = root, L = leaf, S = stem.

Fig. 7

Fig. 8 Phosphorus test after addition of ascorbate to 0% B group. 0% = 0.00 mg B l^{-1}.

R= root, L = leaf, S = stem.

Fig. 8

DISCUSSION

One of the problems incurred with this investigation was keeping the plants from falling into the hydroponics solutions. Obviously, punching smaller holes in the Parafilm should help. However, using a shallower container such as a plastic shoebox or rectangular freezer container with holes drilled in the lid would make it easier to fish out the plants and return them to their proper positions.

Another problem was fungal infection. As this occurred during germination, better surface sterilization of the seeds is needed. A stronger bleach solution or possibly a detergent such as Tween-20 could be used.

The colorimetric phosphorus tests were inconclusive. It is possible that there were no differences in phosphate concentrations between before and after ascorbate supplementation. However, based on the literature, this doesn't seem likely. It may be that the test was not sensitive enough to detect any possible change. Therefore, figures 1 and 2, figures 3 and 4, figures 5 and 6, and figures 7 and 8 show no change between before and after states or even between different boron nutrition concentrations. Perhaps redoing the experiment using a different assay method, e.g. a spectrophotometric test or radioisotopes, might detect a difference in phosphate levels.

REFERENCES

Agarwala, S.C., Sharma, P.N., Chatterjee, C., and Sharma, C.P. (1981) Development and enzymatic changes during pollen development in boron deficient maize plants. *J. Plant Nutr*. 3: 329-336.

Agulhon, H. (1910) Présence et utilité du bore chez les végétaux. *Ann. Inst. Pasteur* 24: 321-329.

Arrigoni, O., Arrigoni-Liso, R., and Calabrese, G. (1977) Ascorbic acid requirement for biosynthesis of hydroxyproline-containing proteins in plants. *FEBS Lett*. 82(1): 135-138.

Barr, R., Böttger, M., and Crane, F.L. (1993) The effect of boron on plasma membrane electron transport and associated proton secretion by cultured carrot cells. *Biochem. Mol. Biol. Int*. 31: 31-39.

Blevins, D.G. and Lukaszewski, K.M. (1998) Boron in plant structure and function. *Annu. Rev. Plant Physiol. Plant Mol. Biol*. 49: 481-500.

Bohnsack, C.W. (1991) Investigating the boron requirement of plants. *Amer. Biol. Teach*. 53(8): 486-488.

Bolaños, L., Esteban, E., de Lorenzo, C., Fernandez-Pascual, M., de Felipe, M.R., Garate, A., and Bonilla, I. (1994) Essentiality of boron symbiotic dinitrogen fixation in pea (*Pisum sativum*) rhizobium nodules. *Plant Physiol*. 104: 85-90.

Bonilla, I., Mergold-Villaseñor, C., Campos, M.E., Sánchez, N., Pérez, H., López, L., Castrejón, L., Sánchez, F., and Cassab, G.I. (1997) The aberrant cell walls of boron-deficient bean root nodules have no covalently bound hydroxyproline-/proline-rich proteins. *Plant Physiol*. 115: 1329-1340.

Bussler, W. (1964) Die bormangelsymptome und ihre entwicklung. *Z. Pflanzenern. Düng. Bodenkde*. 91: 1-14.

Camacho-Christóbal, J.J. and González-Fontes, A., (1999) Boron deficiency causes a drastic decrease in nitrate content and nitrate reductase activity, and increases the content of carbohydrates in leaves from tobacco plants. *Planta* 209: 528-536.

Carpita, N.C. and Gibeaut, D.M. (1993) Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. *The Plant J*. 3 (1): 1-30.

Cakmak, I. and Römheld, V. (1997) Boron deficiency-induced impairments of cellular functions in plants. *Plant Soil* 193: 71-83.

Cassab, G.I. (1998) Plant cell wall proteins. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 49: 281-309.

Conklin, P.L., Norris, S.R., Wheeler, G.L., Williams, E.H., Smirnoff, N., and Last, R.L. (1999) Genetic evidence for the role of GDP-mannose in plant ascorbic acid (vitamin C) biosynthesis. *Proc. Natl. Acad. Sci. USA*. 96(7): 4198-4203.

Cordoba, F., and González-Reyes, J.A. (1994) Ascorbate and plant cell growth. *J. Bioenerg. Biomem*. 26(4): 399-405.

Cordoba-Pedregosa, M. del C., González-Reyes, J.A., and Cordoba, F. (1996) Role of apoplastic and cell-wall peroxidases on the stimulation of root elongation by ascorbate. *Plant Physiol*. 112(3): 1119-1125.

Cosgrove, D.J. (1987) Wall relaxation and the driving forces for cell expansive growth. *Plant Physiol*. 84: 561-564.
Currier, H.B. (1957) Callose substance in plant cells. *Amer. J. Bot*. 44: 478-488.
Darvill, A., McNeil, M., Albersheim, P., and Delmer, D.P. (1980) The primary cell wall of flowering plants. *In* The biochemistry of plants: A comprehensive treatise, Vol.1: The plant cell. Edited by Talbert, N.E. pp. 91-161. Academic Press: New York.
De Cabo, R.C., González-Reyes, J.A., and Navas, P. (1993) The onset of cell proliferation is stimulated by ascorbate free radical in onion root primordia. *Biol. Cell* 77: 231-233
De Tullio, M.C., Paciolla, C., and Arrigoni, O. (1999) Changes in onion root development induced by the inhibition of peptidyl-prolyl hydroxylase and influence of the ascorbate system on cell division and elongation. *Planta* 209(4): 424-430
Dell, B. and Huang, L. (1997) Physiological response of plants to low boron. *Plant Soil*. 193: 103-120.
Devlin, R.M. (1975) Plant physiology, 3rd ed. pp. 6, 138-139. D. Van Nostrand.
Dickinson, D.B. (1978) Influence of borate and pentaerythritol concentrations on germination and tube growth of *Lilium longiflorum* pollen. *J. Amer. Soc. Hort. Sci*. 103(3): 413-416.
Dutta, I. (1987) Boron deficiency and its associated perturbation of nucleotide metabolism in tomato plants. Dissertation. North Texas State University, Denton, Texas, USA.
Dutta, T.R. and McIlrath, W.J. (1964) Effects of boron on growth and lignification in sunflower tissue and organ cultures. *Bot. Gaz*. 125: 89-96.
Eaton, F.M. (1944) Deficiency, toxicity, and accumulation of boron in plants. *J. Agric. Res*. 69: 237-277.
Ferrol, N., Belver, A., Roldán, M., Rodriguez-Rosales, P., and Donaire, J.P. (1993) Effects of boron on proton transport and membrane properties of sunflower (*Helianthus annuus* L.) cell microsomes. *Plant Physiol*. 103: 763-769.
Ferrol, N. and Donaire, J.P. (1992) Effect of boron on plasma membrane proton extrusion and redox activity in sunflower cells. *Plant Sci*. 86: 41-47.
Findeklee, P. and Goldbach, H.E. (1996) Rapid effects of boron deficiency on cell wall elasticity modulus in *Cucurbita pepo* roots. *Bot. Acta*. 109: 463-465.
Fleming, G.A. (1980) Essential micronutrients. I. Boron and molybdenum. *In* Applied soil trace elements. Edited by Davies, B.E. pp.155-165. John Wiley & Sons, New York.
Foyer, C.H., Lelandais, M., Edwards, E.A., and Mullineaux, P.M. (1991) The role of ascorbate in plants, interactions with photosynthesis, and regulatory significance. *In* Active oxygen/oxidative stress and plant metabolism. Edited by Pell, E.J. and Steffen, K.L. American Society of Plant Physiologists, Rockville, Maryland.
Fry, S.C. (1986) Cross-linking of matrix polymers in the growing cell wall of angiosperms. *Annu. Rev. Plant Physiol*. 37: 165-186.
Fry, S.C. (1998) Oxidative scission of plant cell wall polysaccharides by ascorbate-induced hydroxyl radicals. *Biochem. J*. 332(2): 507-515.

Goldbach, H.E. (1985) Influence of boron nutrition on net uptake and efflux of ^{32}P and ^{14}C-glucose in *Helianthus annuus* roots and cell cultures of *Daucus carota*. *J. Plant Physiol*. 118: 431-438.

Goldbach, H.E. (1997) A critical review on current hypotheses concerning the role of boron in higher plants: suggestions for further research and methodological requirements. *J. Trace and Microprobe Tech*., 15(1): 51-91.

Goldbach, H. and Amberger, A. (1986) Influence of boron nutrition on cell wall polysaccharides in cell cultures of *Daucus carota* L. *J. Plant Physiol*. 123: 263-269.

Goldbach, H.E., Hartmann, D. and Rötzer, T. (1990) Boron is required for the stimulation of the ferricyanide-induced proton release by auxins in suspension-cultured cells of *Daucus carota* and *Lycopersicon esculentum*. *Physiol. Plant*. 80: 114-118.

Goldberg, S. (1993) Chemistry and mineralogy of boron in soils. *In* Boron and its role in crop production. Edited by Gupta, U.C. pp. 3-44. CRC Press, Boca Raton, Florida.

González-Reyes, J.A., Döring, O., Navas, P., Obst, G. and Böttger, M. (1992) The effect of ascorbate free radical on the energy state of the plasma membrane of onion (*Allium cepa* L.) root cells: alteration of K^+ efflux by ascorbate? *Biochim. et Biophys. Acta*. 1098: 177-183.

González-Reyes, J.A., Alcaín, F.J., Caler, J.A., Serrano, A., Córdoba, F., and Navas, P. (1994a) Relationship between apoplastic ascorbate regeneration and the stimulation of root growth in *Allium cepa* L. *Plant Sci*. 100: 23-29.

González-Reyes, J.A., Hidalgo, A., Caler, J.A., Palos, R., and Navas, P. (1994b) Nutrient uptake changes in ascorbate free radical-stimulated onion roots. *Plant Physiol*. 104: 271-276.

Gupta, U.C. (1971) Boron and molybdenum nutrition of wheat, barley and oats grown in Prince Edward Island soils. *Can. J. Soil Sci*. 51: 415-422.

Gupta, U.C. (1983) Boron deficiency and toxicity symptoms for several crops as related to tissue boron levels. *J. Plant Nutr*. 6: 387-395.

Gupta, U.C. (1993) Deficiency and toxicity symptoms of boron in plants. *In* Boron and its role in crop production. Edited by Gupta, U.C. pp.147-155. CRC Press, Boca Raton, Florida.

Gupta, U.C. (1993) Deficiency, sufficiency, and toxicity levels of boron in crops. In Boron and its role in crop production. Edited by Gupta, U.C. pp. 137-145. CRC Press, Boca Raton, Florida.

Gupta, U.C. (1993) Sources of boron. *In* Boron and its role in crop production. Edited by Gupta, U. C. pp. 45-51. CRC Press, Boca Raton, Florida.

Hanson, E.J. (1991) Movement of boron out of tree fruit leaves. *Hort. Sci*. 26: 271-273.

Hanson, E.J. and Breen, P.J. (1985a) Xylem differentiation and boron accumulation in 'italian' prune flower buds. *J. Am. Hortic. Sci*. 110: 389-392.

Hanson, E. J. and Breen, P.J. (1985b) Effects of fall boron sprays and environmental factors on fruit set and boron accumulation in "Italian" prune flowers. *J. Am. Hortic. Sci*. 110: 566-70.

Hidalgo, A., González-Reyes, J.A. and Navas, P. (1989) Ascorbate free radical enhances vacuolization in onion root meristems. *Plant, Cell Environ*. 12: 455-460.

Hidalgo, A., Garcia-Herdugo, G., González-Reyes, J. A., Morré, D.J., and Navas, P. (1991) Ascorbate free radical stimulates onion root growth by increasing cell elongation. *Bot. Gaz*. 152: 282-288.

Hirsch, A.M. and Torrey, J.G. (1980) Ultrastructural changes in sunflower root cells in relation to boron deficiency and added auxin. *Can. J. Bot*. 58: 856-866.

Horemans, N., Asard, H., and Caubergs, R. J. (1994) The role of ascorbate free radical as an electron acceptor to cytochrome b-mediated trans-plasma membrane electron transport in higher plants. *Plant Physiol*. 104: 1455-1458.

Hu, H. and Brown, P.H. (1994) Localization of boron in cell walls of squash and tobacco and its association with pectin. *Plant Physiol*. 105: 681-689.

Hu, H. and Brown, P.H. (1996) Phloem mobility of boron is species dependent: evidence for boron mobility in sorbitol-rich-species. *Ann. Bot*. 77: 497-505.

Hu, H., Brown, P.H., and Labavitch, J.M. (1996) Species variability in boron requirement is correlated with cell wall pectin. *J. Exp. Bot*. 47(295): 227-232.

Hu, H., Penn, S.G., Lebrilla, C.B., and Brown, P.H. (1997) Isolation and characterization of soluble boron complexes in higher plants. *Plant Physiol*. 113: 649-655.

Huang, L., Pants, J., Dell, B., and Bell, R.W. (2000) Effects of boron deficiency on anther development and floret fertility in wheat (*Triticum aestivum* L. 'Wilgoyne'). *Ann. Bot*. 85: 493-500.

Ishii, T. and Matsunaga, T. (1996) Isolation and characterization of a boron-rhamnogalacturonan-II complex from cell walls of sugar beet pulp. *Carb. Res*. 284: 1-9.

Jarvis, B.C. and Booth, A. (1981) Influence of indole-butyric acid, boron, myo-inositol, vitamin D_2, and seedling age on adventitious root development in cuttings of *Phaseolus aureus*. *Physiol. Plant*. 53: 213-218.

Jones, J.B.,Jr. (1983) A guide for the hydroponic and soilless culture grower. Timber Press, Portland, Oregon.

Jones, J. B., Jr. (1998) Plant nutrition manual. pp.101-113. CRC Press, New York.

Kask, U. and Rawn, J.D. (1993) General chemistry. pp. 46, 338-339. Wm C. Brown Publishers, Dubuque, Iowa.

Kobayashi, M., Motoh, T., and Azuma, J-I. (1996) Two chains of rhamnogalacturonan II are cross-linked by borate-diol ester bonds in higher plant cell walls. *Plant Physiol*. 110: 1017-1020.

Kouchi, H. and Kumazawa, K. (1975) Anatomical responses of root tips to boron deficiency. I. Effects of boron deficiency on the elongation of root tips and their morphological characteristics. *Soil Sci. Plant Nutr*. 21: 218-228.

Kouchi, Hl and Kumazawa, K. (1976) Anatomical responses of root tips to boron deficiency. III. Effect of boron deficiency on Sub-cellular structure of root tips, particularly on morphology of cell wall and its related organelles. *Soil Sci. Plant Nutr*. 22(1): 53-71.

Lewin, J.C. (1965) The boron requirement of a marine diatom. *Naturwissenschaften.* 70: 1-2.

Lewin, J.C. (1966) Boron as a growth requirement for diatom. *J. Phycol*. 2: 160-163.

Lewis, D.H. (1980) Are there inter-relations between the metabolic role of boron, synthesis of phenolic phyto-alexins and the germination of pollen? *New Phytol*. 84: 261-270.

Liso, R., Calabrese, G., Bitonti, M.B., and Arrigoni, O. (1984) Relationship between ascorbic acid and cell division. *Exp. Cell Res*. 150: 314-320.

Lockman, R.B. (1972) Mineral composition of grain sorghum plant samples. I. Comparative analysis with corn at various stages of growth and under different environments. *Commun. Soil Sci. Plant Anal*., 3: 271

Loomis, W.D. and Durst, R.W. (1992) Chemistry and biology of boron. *BioFactors*. 3(4): 229-239.

Lukaszewski, K.M. and Blevins, D.G. (1996) Root growth inhibition in boron-deficient or aluminum-stressed squash may be a result of impaired ascorbate metabolism. *Plant Physiol*. 112: 1135-1140.

MacKay, D.C., Langille, W.M., and Chipman, E.W. (1962) Boron deficiency and toxicity in crops grown on sphagnum peat soil. *Can. J. Soil Sci*. 42: 302-310.

Marschner, H. (1995) Mineral nutrition of higher plants, 2nd ed. 379-396. Academic Press, San Diego, California.

Mateo, P., Bonilla, I., Fernandez-Valiente, E., and Sanchez-Maeso, E. (1986) Essentiality of boron for dinitrogen fixation in *Anabaena* sp. PCC 7119. *Plant Physiol*. 81: 430-433.

Matoh,T., Kawaguchi, S., and Kobayashi, M. (1996) Ubiquity of a borate-rhamnogalacturonan II complex in the cell walls of higher plants. *Plant Cell Physiol*. 37(5): 636-640.

Matoh, T. (1997) Boron in plant cell walls. *Plant Soil*. 193: 59-70.

Mazé, P. (1915) Determination des elements mineraux rares necessaires as development du mais. *Compt. Rend*. 160: 211-214.

Mazé, P. (1919) Recherche d'une solution purement minerale capable d'assurer l'evolution complete du mais cultive a l'abri des microbes. *Ann. Inst. Pasteur*. 33: 139-176.

McHargue, J.S. and Calfee, R.K. (1933) Further evidence that boron is essential for the growth of lettuce. *Plant Physiol*. 8: 305-313.

Morré, D.J., Navas, P., Penel, C., and Castillo, F.J. (1986) Auxin-stimulated NADH oxidase (semidehydroascorbate reductase) of soybean plasma membrane: role in acidification of cytoplasm? *Protoplasma* 133: 195-197.

Mozafar, A. (1987) Effect of boron on ear formation and yield components of two maize (*Zea mays* L.) hybrids. *J. Plant Nutr*. 10: 319-332.

Mozafar, A. (1989) Boron effect on mineral nutrients of maize. *Agron. J*. 81: 285-290.

Nielson, F.H., Hunt, C.F., Mullen, L.M., Hunt, J.R. (1987) Effect of dietary boron on mineral, estrogen, and testosterone metabolism in postmenopausal women. *FASEB J*. 1: 394-397.

Nishikimi, M., Fukuyama, R., Minoshima, S., Shimizu, N., and Yagi, K. Cloning and chromosomal mapping of the human nonfunctional gene for L-gulono-gamma lactone oxidase, the enzyme for L-ascorbic acid biosynthesis missing in man. *J. Biol. Chem*. 269(18): 13685-13688.

Nobel, P.S. (1991) Physicochemical and environmental plant physiology. pp. 33-35. Academic Press Inc., New York, USA.
Noctor, G. and Foyer, C.H. (1998) Ascorbate and glutathione: keeping active oxygen under control. *Annu. Rev. Plant Physiol. Plant Mol. Biol*. 49: 249-279.
Norrish, K. (1975) Geochemistry and mineralogy of trace elements. *In* Trace elements in soil-plant-animal systems. Edited by Nicholas, D.J.D. and Egan. pp. 55-67. Academic Press, New York.
Odhnoff, C. (1957) Boron deficiency and growth. *Physiol. Plant*. 10: 984-1000.
Ohki, R. Mn and B effect on micronutrients and P in cotton. *Agron. J*. 67: 204-207.
Palser, B.F. and McIlrath, W.J. (1956) Responses of tomato, turnip and cotton to variations in boron nutrition. II. Anatomical responses. *Bot.Gaz*. 118: 53-71.
Pollard, A.S., Parr, A.J., and Loughman, B.C. (1977) Boron in relation to membrane function in higher plants. *J. Exp. Bot*. 28: 831-841.
Purvis, E.R. and Carolus, R.L. (1964) Nutrient deficiencies in vegetable crops. *In* Hunger signs in crops, a symposium. Edited by Sprague, H.B. pp. 245-186. McKay, New York.
Ram, S., Bist, L.D., and Sirohi, S.C. (1989) Internal fruit necrosis of mango and its control. *Acta Hort*. 231: 805-813.
Reisenauer, H.M., Walsh, L.M., and Hoeft, R.G. (1973) Testing soils for sulfur, boron, molybdenum, and chlorine. *In* Soil testing and plant analysis. Edited by Walsh, L.M. and Beaton, J.O. pp.173. Soil Science Society of America, Madison, Wisconsin,
Rerkasem, B., Netsangtip, R., Bell, R.W., and Loneragan, J.F. (1988) Comparative species responses to boron on a Typic Tropaqualf in Northern Thailand. *Plant Soil*. 106: 15-21.
Rerkasem, B., Bell, R.W., Lodkaew, S., and Loneragan, J.F. (1993) Boron deficiency in soybean (*Glycine max* L. Merr.), peanut (*Arachis Hypogaea* L.), and black gram (*Vigna mungo* L. Hepper): symptoms in seeds and differences among soy bean cultivars in susceptibility to boron deficiency. Plant Soil. 150(2): 289-294.
Robbertse, P.J., Lock, J.J., Stoffberg, E., and Coetzer, L.A. (1990) Effect of boron on directionality of pollen tube growth in *Petunia* and *Agapanthus*. *S. Afr. J. Bot*. 56: 487-492.
Robertson, G.A. and Loughman, B.C. (1974) Reversible effects of boron on the absorption and incorporation of phosphate in *Vicia faba* L. *New Phytol*. 73: 291-298.
Roldán, m., Belver, A., Rodríguez-Rosales, P., Ferrol, N., and Donaire, J.P. (1992) *In vivo* and *in vitro* effects of boron on the plasma membrane proton pump of sunflower roots. *Physiol. Plant*. 84: 49-54.
Ryan, J., Miyamoto, S, and Stroehlein, J.L. (1977) Relation of solute and sorbed B to the B hazard in irrigation water. *Plant Soil*. 47: 253-256.
Schmucker, T. (1934) Über den einfluss von borsäure auf pflanzen, insbesondere keimende pollenkörner. *Planta* 23: 264-283.
Schon, M.K., Novacky, A., and Blevins, D.G. (1990) Boron induces hyperpolarization of sunflower root cell membranes and increases membrane permeability to K^+. *Plant Physiol*. 93: 566-571.
Scott, C.E. (1944) Boron nutrient of the grape. *Soil Sci*. 57: 55-65.

Shelp, B.J. (1988) Boron mobility and nutrition in broccoli (*Brassica oleracea* var. italica. *Ann Bot*. 61: 83-91.
Shelp, B.J. and Shattuck, V.I. (1987a) Boron nutrition and mobility, and its relation to the elemental composition of greenhouse grown root crops. I. Rutabaga. *Commun. Soil Sci. Plant Anal*. 18(2): 187-201.
Shelp, B.J., Shattuck, V.I., and Proctor, J.T.A. (1987b) Boron nutrition and mobility, and its relation to the elemental composition of greenhouse grown root crops. II. Radish. *Commun. Soil Sci. Plant Anal*. 18(2): 203-219.
Shkolnik, M. YA. (1984) Trace elements in plants. pp. 68-109. Elsevier, New York.
Skok, J. (1941) Effect of boron on growth and development of the radish. *Bot. Gaz*. 103: 280-294.
Smilde, K.W. and van Luit, B. (1970) Boron Deficiency in roots. *Bedrijfsontwikkeling*. 1: 30
Smirnoff, N. (1996) The function and metabolism of ascorbic acid in plants. *Ann. Bot*. 78: 661-669.
Sommer, A.L. and Lipman, C.B. (1926) Evidence on the indispensable nature of zinc and boron tho higher green plants. *Plant Physiol*. 1: 231-249.
Sommer, A. and Sorokin, H. (1928) Effects of the absence of boron and of some other essential elements on the cell and tissue structure of the root tips of *Pisum sativum*. *Pl. Physiol. Lancaster*. 3: 237-260.
Spur, A.R. (1957) The effect of boron on cell-wall structure in celery. *Am. J. Bot*. 44: 637-650.
Taiz, L. and Zeiger, E. (1991) Plant physiology. p. 48. The Benjamin/Cummings Publishing Company, Inc., Redwood City, California.
Takahama, U. and Oniki, T. (1992) Regulation of peroxidase-dependent oxidation of phenolics in the apoplast of spinach leaves by ascorbate. *Plant Cell Physiol*. 33(4): 379-387.
Teasdale, R.D. and Richards, D.K. (1990) Boron deficiency in cultured pine cells. Quantitative studies of the interaction with Ca and Mg. *Plant Physiol*. 93: 1071-1077.
Van de Venter, H.A. and Currier, H.B. (1977) The effect of boron deficiency on callose formation and ^{14}C translocation in bean (*Phaseolus vulgaris* L.) and cotton (*Gossypium hirsutum* L.) *Amer. J. Bot*. 64(7): 861-865.
Waldron, K.W. and Selvendran, R.R. (1990) Composition of the cell walls of different asparagus (*Asparagus officinalis*) tissues. *Physiol. Plant*. 80: 568-575.
Warington, K. (1923) The effect of boric acid and borax on the broad bean and certain other plants. *Ann. Bot*. 37: 629-672.
Wear, J.I. and Patterson, R.M. (1962) Effect of soil pH and texture on the availability of water-soluble B in the soil. *Soil Sci. Soc. Am. Proc*. 26: 344-345.
Wheeler, G.L., Jones, M.A., and Smirnoff, N. (1998) The biosynthetic pathway of vitamin C in higher plants. *Nature*. 393: 365-369.
Woodruff, J.R. (1979) Soil boron and soybean leaf boron in relation to soybean yield. *Commun. Soil Sci. Plant Anal*. 10: 941.
Yamaguchi, M., Howard, F.D., and Minges, P.A. (1958) Brown checking of celery, a symptom of boron deficiency. III. Effects of potassium, nitrogen, and boron in

culture solutions on the physiological disorder and nutrient uptake. *Proc. Am. Soc. Hortic. Sci*. 71: 455.

Zablackis, E., Huang, J., Müller, B., Darvill, A.G., and Albersheim, P. (1995) Characterization of the cell wall polysaccharides of *Arabidopsis thaliana* leaves. *Plant Physiol*. 107: 1129-1138.